3. THE CANCER PROCESS AND PATHOPHYSIOLOGY OF COLORECTAL CANCER: GENERAL CONCEPTS

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Cancers arise as a result of a multistep process involving multiple mutations in different genes. Cancer develops as a result of interaction between genetic susceptibility and protective or promoting role of environmental factors, such as diet and lifestyle.

The genetic changes, which may lead to clinical colorectal cancer, have been intensively investigated for the last thirty years. It has been noticed that colon cancer is usually observed in one of three major patterns: sporadic, inherited or familial.

The most frequent, sporadic, accounts for about 70% of colorectal cancer cases in the population. Sporadic colorectal cancer occurs in persons without familial or inherited predisposition usually over 50 years of age. It is attributed to dietary and environmental factors cumulated with the age.

Inherited colorectal cancer covers two main groups of cancers: with and without polyps, which are (or not) major manifestations of the disease. The polyposis syndromes are divided into familial adenomatous polyposis and the hamartomatous polyposis syndromes. The non-polyposis predominant syndromes include hereditary non-polyposis colorectal cancer (HNPCC) (Lynch syndrome I) and the cancer family syndrome (Lynch syndrome II).

Familial colon cancer is recognised when colon cancer develops too frequently to be considered sporadic, but not in a pattern characteristic for inherited syndrome (1).

The hereditary cancer is a result of the mutations presented in the parent cells and is transmitted from the mother or father as inherited defect.

More common, sporadic colorectal cancer develops as a result of spontaneous mutation occurring in a somatic cell(s) during the growth and development. If the mutation results in uncontrolled proliferation, the development of cancer is observed. Sporadic colorectal cancer occurs when accumulation of multiple mutations is present (2).

The natural history of colorectal cancer

Data from clinical and pathological studies showed that most human colorectal cancers arise from adenomas (3, 4). However, small part of adenomas progresses into cancer. Only about 10% of all of adenomas, at least 1 cm in diameter, develop into cancer during the following 10 years (5). It is not clear how much time takes progression from normal

mucosa to polyp. Typically, sporadic colon cancer appears at older age. It suggests that it needs more than 10–20 years for the development of the polyp, but there are some hereditary colorectal cancers that typically appear at second or third decade of life. In this case, the progression is much more rapid.

To develop cancer, three main types of genes are usually mutated: oncogenes, tumour suppressor genes, and mismatch repair genes. Oncogenes are normal genes responsible for the stimulation of controlled cellular proliferation (6). The mutation of these genes leads to uncontrolled cellular proliferation. Tumour suppressor genes (antioncogenes) are responsible for inhibition of cell cycle and promotion of apoptosis. If the function of the latter two is disturbed, the uncontrolled proliferation follows. However, one functioning allele of these genes is enough to control the cell cycle (7). Mismatch repair genes are genes coding enzymes responsible for monitoring newly formed DNA and correcting replication errors (8). Defective MMR genes are associated with phenotype called mutator phenotype. The accumulation of replication errors throughout genome increases the probability of the mutation in important regulatory genes and may lead to cancer (9).

The genetics of hereditary colorectal cancers

The hereditary colorectal cancers are familial adenomatous polyposis and hereditary non-polyposis colorectal cancers (HNPCC).

Familial adenomatous polyposis is an autosomal dominantly inherited disease, which occurs in 1 per 7000 individuals. The penetrance of the disease is almost 100%. The disease develops most frequently in the second or third decade of life, presenting with a number of polyps throughout the whole large bowel.

Molecular studies on familial adenomatous polyposis (FAP) originally suggested that the germline mutation responsible for the disease is the mutation in the tumour suppressor gene, adenomatous polyposis coli (APC), localised on chromosome 5q (10). However, later observations showed that up to 30% of patients with the diagnosis of FAP might show no APC mutations (11). It has been suggested that in those patients the presence of bi-allelic mutation of the MYC gene is responsible for the development of FAP (12).

Hereditary non-polyposis colorectal cancer is also autosomal dominant disease. Typically, it appears at early age and predominantly affects proximal colon. HNPCC tumours have typical pathologic features like lymphocytic infiltration, high mucinous content and poor differentiation. Clinically, HNPCC is divided into Lynch I and Lynch II syndrome. Lynch I syndrome is characterised by the changes observed only in the colon and Lynch II syndrome is diagnosed when other, extracolonic localizations, like endometrial, ovarian, urinary, pancreatic, gastric, small bowel or brain cancer(s) are observed (13). The penetrance of the HNPCC is about 80%.

The genome analysis of the HNPCC patients showed that most of these cancers presented short repetitive DNA sequences, the so called microsatellites (14). The disease was attributed to the mutations in DNA mismatch repair (MMR) genes, *hMLHl and hMSH2* (15). There were also discovered additional genes responsible for the development of HNPCC, i.e., *hPMS1*, *hPMS2*, *hMLH3*, *hMSH3*, *hMSH6*, and *EXO1* (16, 17, 18). The most common genes observed among patients with HNPCC are *hMLH1* and *hMSH2*. Mutations of these two genes are observed in about 90% known mutations in

HNPCC. The mutations of MMR lead to accumulations of replication errors and microsatellite instability (MSI) phenotype (19). High level of MSI (MSI-H) is observed typically when mutations in *hMLH1* and *hMSH2* are both present.

The genetics of sporadic colorectal cancer

The genetic changes, which may lead to sporadic colorectal cancer were first described by Fearon and Vogelstein (2) as the multistep process that might be observed in the pathway from the healthy tissue to the cancerous tissue (Fig. 3.1). It was suggested that colorectal cancer tumours develop as a result of the accumulation of activated oncogenes and inactivated tumour suppressor genes.

The first noticed mutation in the pathway is the mutation in the tumour suppressor gene APC that was observed in 50% of cancer cases and in about 30% of adenomas. It was suggested that the gene might be responsible for the initiation of the process. The APC gene is thought to modulate the beta-catenin protein, which regulates cell signal

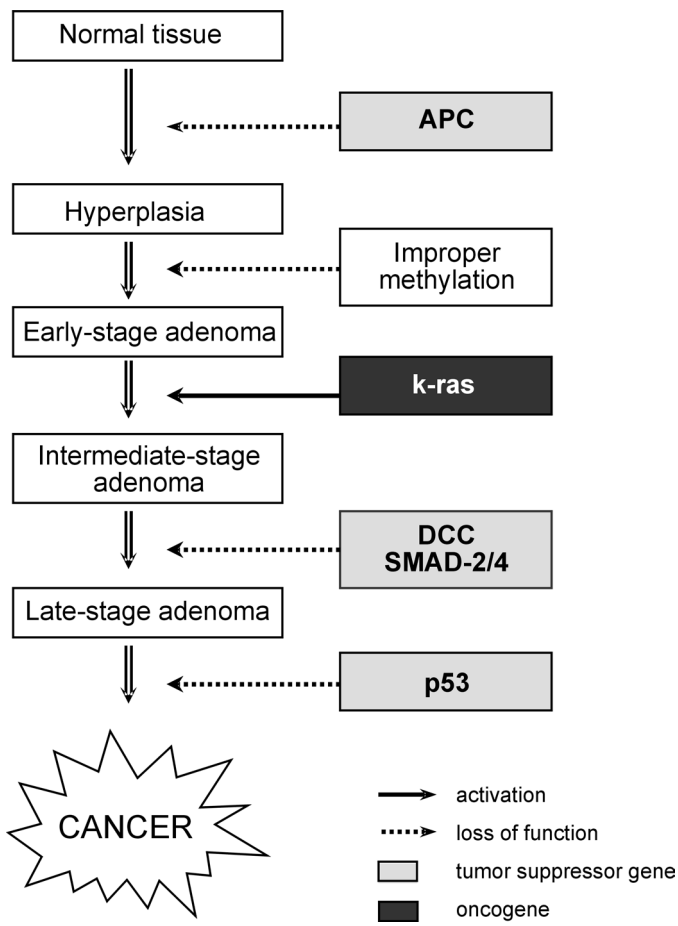


Figure 3.1. Multistep process of tumorigenesis in the “suppressor” pathway

transduction and growth (20). APC mutations play key role in early cell transformation, and thus, the APC gene is sometimes called "gatekeeper" gene (21).

The oncogene *k-ras* is another gene involved in the carcinogenesis of colorectal cancer. The gene codes for the binding protein that acts as one-way switch for extracellular growth signals. Improper activation of *k-ras* leads to change of related protein that results in continuous stimulation of the cell to grow. Mutations of *k-ras* are detected in up to 50% of cases of sporadic colorectal cancer.

Another gene responsible for colorectal cancer is the DCC (deleted in colorectal adenoma) (22). The *DCC* is localized on 18q and it encodes a protein that plays role in cell to cell interactions. Though deletions of *DCC* were found in over 70% of tumours, inactivating mutations were not found in the residual *DCC* allele (4). Moreover, some investigators discovered other deleted genes on the chromosome 18q, *SMAD-2* and *SMAD-4*, and these genes seem to have clearer characteristic of tumour suppressors (23).

The next gene in the pathway is *p53*. It is tumour suppressor gene responsible for the arrest of cell cycle in the G_1 stage to facilitate repair of DNA and to induce apoptosis, if required. Hence, it is sometimes called "guardian of the genome." The inactivation of the *p53* is observed in about 75% of sporadic colorectal cancers.

There is an agreement, however, that not appropriate chronological order of appearance, but accumulation of changes is responsible for the development of tumour. Tumours evolving through inactivating mutations of tumour suppressor genes and accompanying mutations of oncogenes or losses of adequate alleles (originally termed "loss of heterozygosity" (LOH) due to chromosomal losses observed on chromosomes 5q, 17p and 18q among patients with sporadic colorectal cancer (2)) represent the "chromosomal instability" (CIN) also called a "suppressor" pathway (24).

Observation of sporadic colorectal cancers showed that only about 50% of tumours had features typical for "suppressor" pathway. In 14% of tumours the high microsatellite instability (MSI-H) phenotype was observed (25). The MSI-H phenotype is typically observed among HNPCC, and was described as another potential mechanism of tumorigenesis in colorectal cancer called a "mutator" pathway (26) [see above]. Above 3% of tumours had both "suppressor" and "mutator" characteristic, thus about 40% of sporadic colorectal cancers might progress through other molecular mechanisms.

The third proposed pathway of tumorigenesis in colorectal cancer involved transcriptional silencing of selected genes, and it has been termed "CpG island methylator phenotype" (CIMP) (27). This phenotype is different from age-related (type A) methylation that occurs with the ageing. The mechanism of epigenetic gene silencing, by methylation of the particular gene promoter, leads to lack of activity of tumour suppressor genes in some cancers (28). There is also evidence that hypermethylation of promoter region of *hMLH1* leads to microsatellite instability (MSI) in non-hereditary colorectal cancers (29). It has been found that both alleles of *hMLH1* were hypermethylated in five out of six MSI colon cancer cell lines that lacked identifiable mismatch repair gene mutations (30). Increasing attention on the contribution of epigenetics to tumorigenesis resulted in discovering other genes, which silencing by mypermethylation lead to the loss of key regulatory functions (31).

Alternative model of tumorigenesis in colorectal cancer suggests that some tumours may arise as a result of inhibition of apoptosis and subsequent inactivation of the DNA

repair system. It has been shown that aberrant crypt foci (ACF) (the earliest microscopic lesions of dysplasia) are precursor lesions of colorectal adenomas and cancer. There are data suggesting that some sporadic colorectal cancers arise as a result of *k-ras* mutations that lead to ACF and adenomas without inhibition of the APC signalling pathway (32). This model of tumorigenesis probably accounts for a significant proportion of serrated adenomas (33) and is termed "serrated" pathway.

The increasing knowledge on genetic changes in colorectal cancer tumorigenesis gives the opportunity for the development of molecular tools for prevention and early diagnosis. Some molecular DNA-based stool tests are under investigation in terms of their sensitivity and specificity. The main candidates for screening are single gene tests of mutations of *k-ras* and APC and multiple gene test, which used a panel of three genes: *p53, k-ras* and microsatelite marker BAT-26 (34, 35). More investigations, however, are

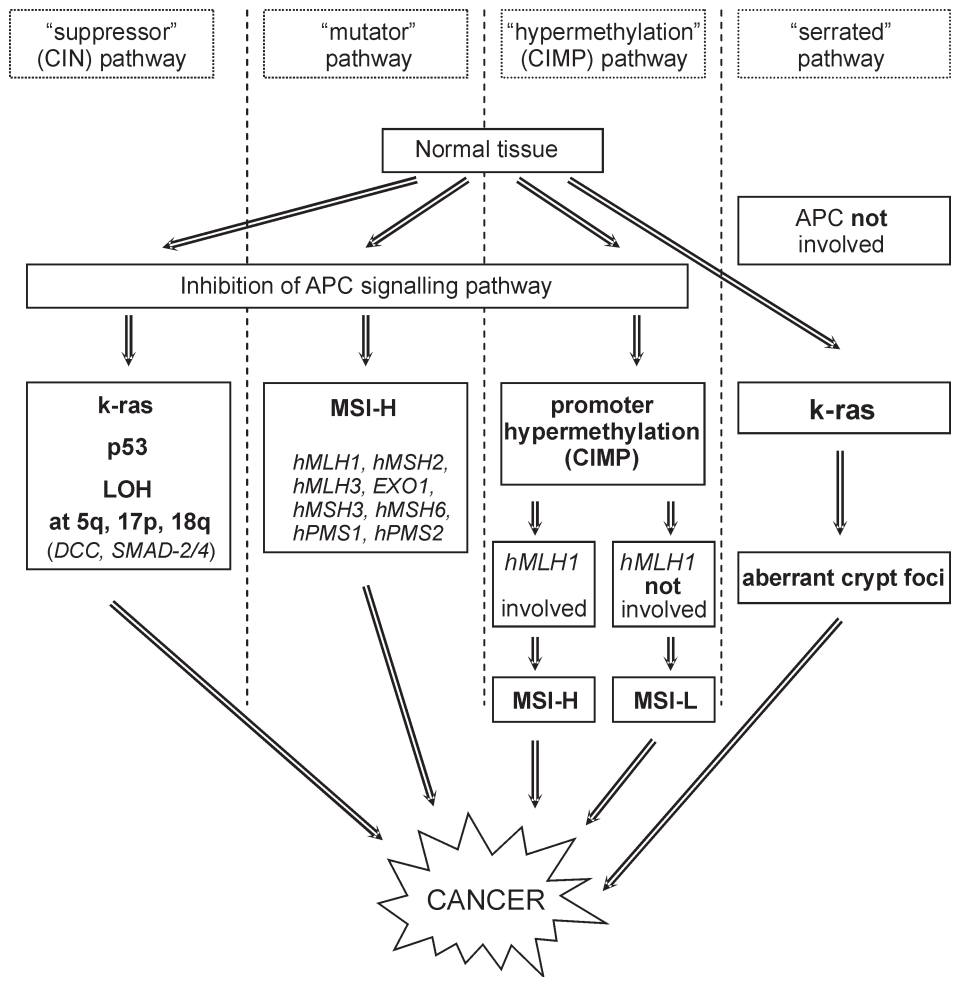


Figure 3.2. Multiple pathways to colorectal cancer

required to assess the real validity of these new screening procedures, their cost-effectiveness and comparability with conventional screening tests.

Risk factors

About 60% of colorectal cancers are attributable to improper dietary habits, the remaining 35% to genetic predispositions and 5% to environmental factors (36). The main risk factors for colorectal cancer are low consumption of fruits and vegetables (which provide dietary fiber, carotenoids and other antioxidative vitamins, isoflavons, flavonoids, polyphenols, selenium and folate), high consumption of red meat, high amount of saturated fatty acids, and alcohol, as well as low level of physical activity (37). Possible mechanisms responsible for the effect and available evidence are described in chapter 4 (*Causes of colorectal cancer*).

There are several medical conditions that have effect on colorectal cancer risk. Some of them, like the use of non-steroidal anti-inflammatory drugs and hormone replacement therapy decrease it, while others, especially certain diseases, may have the opposite effect (Table 3.1).

Table 3.1. Risk factors for colorectal cancer

❑ Intestinal polyp(s) ❑ Chronic inflammatory bowel disease ♦ Ulcerative colitis ♦ Crohn's disease ❑ Diabetes mellitus ❑ Cholecystectomy	**Hereditary syndromes** ❑ Familial adenomatous polyposis ❑ Gardner syndrome ❑ Hereditary non-polyposis colorectal cancer syndrome ❑ Oldfield syndrome ❑ Turcot syndrome ❑ Peutz-Jeghers syndrome ❑ Juvenille polyposis ❑ Cowden disease ❑ Bannayan-Ruvalcaba syndrome ❑ Li-Fraumeni syndrome ❑ Bloom syndrome

Non-modifiable risk factors of colorectal cancer (CRC):

1. Age – the risk increases with the age; more than 90% of patients are old over 50 years at the time of CRC diagnosis.
2. Family history of colorectal cancer – persons with a history of colorectal cancer in the first-degree relatives run twice as high risk of CRC, and the risk is higher if CRC was diagnosed in the relative aged below 60 years or if two or more first-degree relatives were diagnosed to have CRC.
3. Race – the highest CRC risk was observed among African Americans.
4. Ethnic – Ashkenazi Jews run the highest CRC risk in the world due to the presence of several gene mutations (e.g., I1307K APC mutation).

Signs and symptoms

In general, there are no early symptoms of the CRC. Table 3.2 demonstrates a variety of symptoms reported by the patients that may be associated with colorectal cancer.

Table 3.2. Symptoms in colorectal cancer

❑ Change in bowel habits ❑ Diarrhea ❑ Constipation ❑ Unfinished bowel movement feeling ❑ Blood in stool ❑ Narrower stools ❑ Gastrointestinal bleeding ❑ Abdominal discomfort	❑ Abdominal bloating ❑ Abdominal fullness ❑ Abdominal cramps ❑ Loss of appetite ❑ Weight loss ❑ Extreme tiredness ❑ Vomiting ❑ Anemia

There are no pathognomonic symptoms as well. All above mentioned manifestations may accompany many other gastrointestinal disorders, however, if any of these appear, it is necessary to contact general practitioner for physical examination and necessary diagnostic procedures.

Diagnosis

Routine diagnostic procedures in colorectal cancer are presented in Table 3.3.

Table 3.3. Diagnostic procedures in colorectal cancer (38, 39, 40, 41, 42, 43, 44)

Diagnostic procedure	Sensitivity[$] (range) [in %]	Specificity[$] (range) [in %]
❑ Digital rectal examination*	76	92
❑ Rectoscopy*	78	84
❑ Flexible sigmoidoscopy	90 (80–95)	95 (90–100)
❑ Colonoscopy	95 (85–95)	100
❑ Virtual colonoscopy (CT)	65-90 (58–96)	86-89 (81–92)
❑ Fecal occult blood test (FOBT) unrehydrated	33 (20–40)	97 (95–99)
❑ Fecal occult blood test (FOBT) rehydrated	60 (40–65)	90 (85–95)
❑ Double-contrast barium enema	70 (60–90)	86 (80–98)
❑ Fecal immunochemical tests	61–91	97–98
❑ Genetic (MSI) testing**	81 (73–89)	92 (90–94)

[$] – for cancer
* – for rectal cancer
** – genetic testing should be considered as one part of the clinical evaluation of patients who are suspected of having inherited colon cancer syndromes

The final diagnosis of colorectal cancer is based on histopathological examination of suspected tissue obtained by biopsy.

There are three main types of colorectal cancer:

1) sporadic colorectal cancer – diagnosed among persons without familial or inherited predisposition,
2) inherited colorectal cancer – covers any of inherited cancers, which are divided into colorectal cancer with polyps and without polyps:

Without polyps	With polyps
❑ Hereditary Non-Polyposis Colorectal Cancer (HNPCC)* • AC-1 group A (with MMR deficiency; showed increased incidence of extracolonic cancers; previously Lynch II) • AC-1 group B (without MMR deficiency; showed lower incidence of extracolonic cancers; previously Lynch I)	❑ Familial Adenomatous Polyposis (FAP) ❑ Attenuated Familial Adenomatous Polyposis (AFAP) ❑ Mixed Polyposis Syndrome ❑ Ashkenazi I1307K colon cancer ❑ Hereditary Breast and Colorectal Cancer (HBCC; CHEK2) ❑ Hamartomatous Polyposis Syndrome • Peutz-Jeghers syndrome • Familial Juvenile Polyposis • Cowden's disease • Bannayan-Ruvalcaba-Riley syndrome

* new classification proposed by Lindor et al. (45) and based on the knowledge about MMR mutations and their causal significance in the Lynch syndrome

3) familial colon cancer – recognized when colon cancer develops too frequently to be considered sporadic, but not in a pattern characteristic for inherited syndrome.

In 1991 clinical Amsterdam criteria were proposed for HNPCC. Later on, after the researchers had found out that mutations of the MMR genes are causing HNPCC, the newer, Bethesda criteria were developed that incorporated the pre-existing Amsterdam guidelines (see Table 3.4 below).

Table 3.4. Clinical criteria for Hereditary Non-Polyposis Colorectal Cancer (HNPCC) (46, 47)

Amsterdam (I) criteria: At least three relatives with histologically confirmed colorectal cancer and all of the following: 1) one affected person is a first-degree relative of the other two affected persons 2) two successive generations affected 3) at least one of the relatives with colorectal cancer diagnosed before 50 years of age 4) Familial Adenomatous Polyposis has been excluded
Modified Amsterdam (II) criteria: At least three relatives with histologically confirmed HNPCC-associated* cancer and all of the following: 1) one affected person is a first-degree relative of the other two affected persons 2) two successive generations affected 3) at least one of the relatives with colorectal cancer diagnosed before 50 years of age 4) Familial Adenomatous Polyposis has been excluded

Bethesda criteria:

The Amsterdam criteria or one of the following:

1) two cases of HNPCC-associated* cancer diagnosed in one patient, including synchronous or metachronous cancer
2) diagnosis of colorectal cancer and a first-degree relative with HNPCC-associated* cancer and/or colonic adenoma (one case of cancer diagnosed before 45 years of age and adenoma diagnosed before 40 years of age
3) colon or endometrial cancer diagnosed before 45 years of age
4) right sided colon cancer that has an undifferentiated pattern (solid-cribriform) or signet-cell histopathologic characteristics diagnosed before 45 years of age
5) adenomas diagnosed before 40 years of age

* HNPCC-associated cancers include: colorectal, endometrial, stomach, ovarian, pancreas, ureter or renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumours, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

Screening

It is well known that colorectal cancer screening is effective, improves surveillance and provides life-saving benefits. The type of screening procedure and frequency of examination is related to the risk of developing colorectal cancer in the future, and this depends mainly on the individual genetic predisposition.

Risk	Screening method	Age to begin screening	Interval
Classical FAP (48)	Sigmoidoscopy[1]	10–12	2 years[1]
Attenuated FAP (48)	Colonoscopy	18–20	2 years[1]
HNPCC[2] (49)	Colonoscopy	20–25	1–2 years
Familial clustering of colorectal cancer without evidence of MSI[3] (49)	Colonoscopy	45–50 or 5–10 before age at diagnosis of first CRC in family	3–5 years
≥ 2 first-degree relatives with colorectal cancer or adenomatous polyps at age < 60	Colonoscopy	40 or 10 before age at diagnosis of first CRC in family	3–5 years
First-degree relative with colorectal cancer or adenomatous polyp at age ≥ 60	FOBT sigmoidoscopy FOBT + sigmoidoscopy4 DCBE Colonoscopy	40 40 40 40 40	1 year 5 years 1 and 5 years 5–10 years 10 years
Average risk	FOBT sigmoidoscopy FOBT + sigmoidoscopy DCBE Colonoscopy	50 50 50 50 50	1 year 5 years 1 and 5 years 5–10 years 10 years

FAP – familial adenomatous polyposis; HNPCC – hereditary non-polyposis colorectal cancer; MSI – microsatellite instability; CRC – colorectal cancer; DCBE – double-contrast barium enema.

[1] once adenomas are detected annual colonoscopy should be performed until colectomy is planned,

[2] at the age 30–35 other screening methods are recommended for extracolonic localisation, like gynaecological examination, transvaginal ultrasound, aspiration biopsy, gastroduodenoscopy, abdominal ultrasound, and if urinary tract cancer runs in the family also urinalysis and urine cytology,

[3] Amsterdam positive families,

[4] combined testing (e.g., FOBT annually and sigmoidoscopy every 5 years) is preferred over either annual FOBT or sigmoidoscopy every 5 years alone.

Pathology

Over 95% of colorectal cancers are adenocarcinomas. There are also other, however, very rare cancers observed in colon and rectum, like epidermoid (1.5%), other than adenocarcinoma specified cancers (13%) (carcinoid, small cell carcinoma, undifferentiated carcinoma), and sarcomas (0.1%) (leiomyosacromas, angiosarcomas, lyposarcomas, and fibrosarcomas) (50).

The staging of colorectal cancer is based on Duke's classification (Fig. 3.3) modified later by Astler-Coller:

- Duke A – tumour penetrates into the mucosa of the bowel but not infiltrates muscularis propria,
- Duke B1 – tumour penetrates into but not through the muscularis propria,
- Duke B2 – tumour penetrates into and through the muscularis propria, there is no pathologic evidence of metastatic cells in the lymph nodes,
- Duke C1 – tumour penetrates into but not through the muscularis propria, but there is pathologic evidence of metastatic cells in the lymph nodes,
- Duke C2 – tumour penetrates into and through the muscularis propria, and there is pathologic evidence of metastatic cells in the lymph nodes,
- Duke D – tumour has spread beyond the lymph nodes, to distant organs.

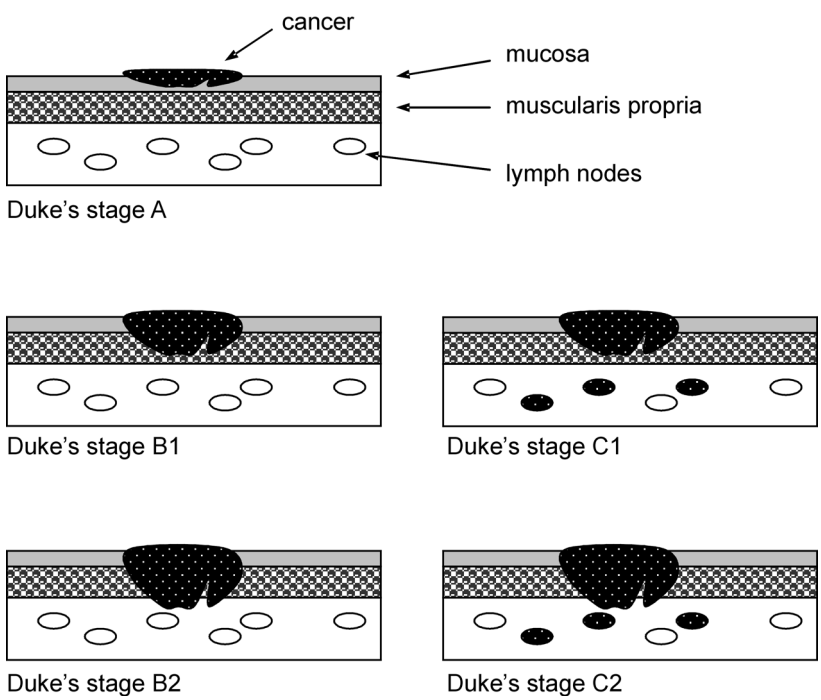


Figure 3.3. The Duke's classification of colorectal cancer

The American Joint Committee on Cancer (AJCC) introduced the system classifying tumours into the four (in fifth edition) and into seven (in sixth edition) stages, which depend on the tumour (T), lymph nodes (N) and metastases (M) features:

Tumour (T)	Lymph nodes (N)	Metastasis (M)
T1 – tumor invades submucosa T2 – tumor invades muscularis propria T3 – tumor invades through the muscularis propria into the subserosa, or into the pericolic or perirectal tissues T4 – tumor directly invades other organs or structures, and/or perforates*	N0 – no regional lymph node metastasis N1 – metastasis in 1 to 3 regional lymph nodes N2 – metastasis in 4 or more regional lymph nodes	M0 – no distant metastasis M1 – Distant metastasis present

* The most likely organs to experience metastasis from colorectal cancer are the lungs and liver.

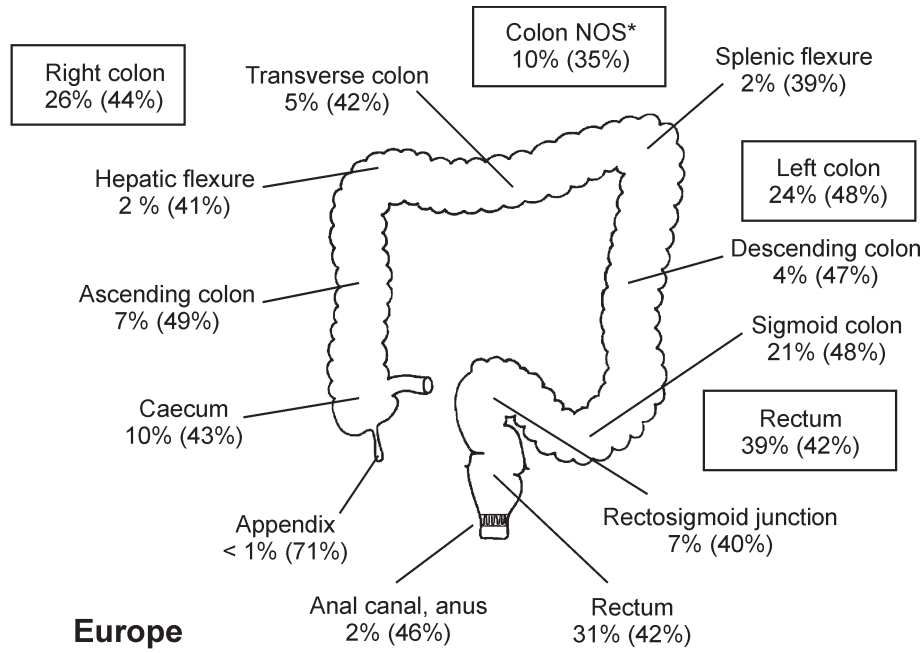
Stages as defined by the American Joint Committee on Cancer (AJCC) are as follows:

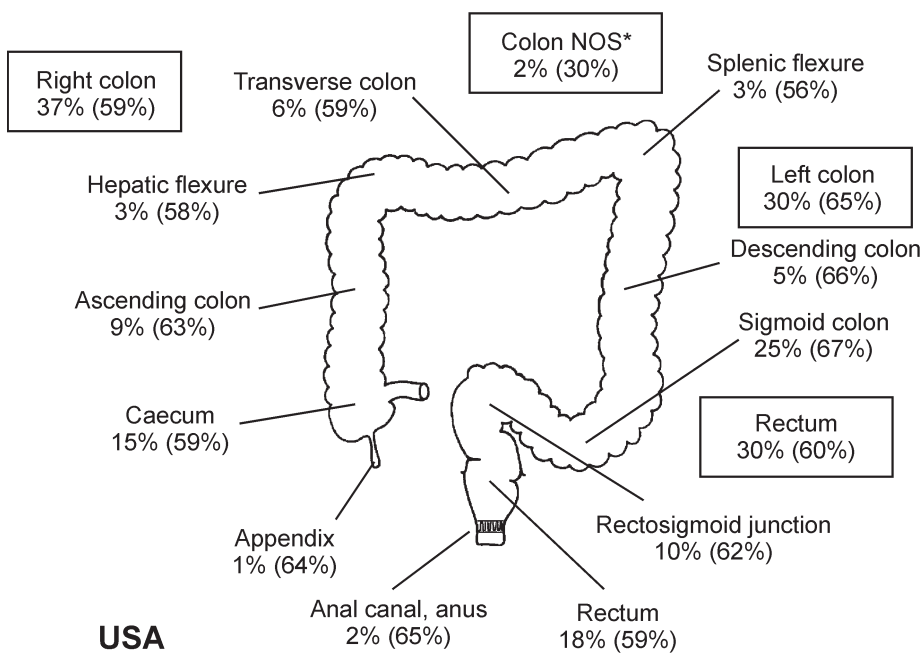
	T	N	M
AJCC – 5th edition			
I	T1 or T2	N0	M0
II	T3 or T4	N0	M0
III	any T	N1	M0
IV	any T	any N	M1
AJCC – 6th edition			
I	T1 or T2	N0	M0
IIa	T3	N0	M0
IIb	T4	N0	M0
IIIa	T1 or T2	N1	M0
IIIb	T3 or T4	N1	M0
IIIc	any T	N2	M0
IV	any T	any N	M1

These classifications are used to stratify patients in terms of predicted survival, to support the decision making on the most effective treatment, to prognose, and to evaluate cancer control measures (51).

Prognosis and survival

The effectiveness of treatment of colorectal cancer depends on the stage of the disease at the time of diagnosis. The analysis of the data from the Surveillance, Epidemiology and End Results (SEER) program, a large US cancer registry, from the year 1991 through the end of 2000 showed, that 5-year survival for patients at stage I (6-th AJCC) was about 93%, at stage IIa – 85%, IIb – 72%, IIIa – 83%, IIIb – 64%, and at stage IIIc – 44%. The lowest survival rate was observed for those patients, who were at stage IV. In that





* NOS = not otherwise specified; 1% of cases was overlapping sites

Figure 3.4. Distribution of colorectal cancer by tumour site and 5-year survival rates (in parenthesis) by regions (USA and Europe)

group, the 5-year survival only slightly exceeded 8% (52). The prognosis depends also on the histologic subtype of the cancer. The worse prognosis was observed for signet ring cell carcinomas (5-year survival: 36%). Survival rate depends not only on stage of the cancer, but also on tumour localization in the large intestine. In the SEER (1991–2000) cohort the best 5-year survival was observed among patients with tumours located in the sigmoid colon (70%). Figure 3.4 shows difference between the USA and Europe for colorectal cancer localization and survival rates (50).

It is worth to remember that there are a lot of demographic, clinical and social conditions, which influence the effectiveness of screening, diagnosis, treatment, and finally the survival rate.

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